REMARKS

The specification has been amended to correct an obvious typographical error regarding the abbreviation of peripheral blood mononuclear cells. No new matter was added.

Claims 1-32 were pending in the application, claims 1-4, 9-12, 17, 29-32 were canceled, claims 19-28 are withdrawn from consideration, claims 5, 6, 7, 13, 15, 16, and 18 were amended and new claim 33 was added to claim additional aspects of the invention. Support for new claims can be found throughout the specification and, for example, at page 12, line 17-28. No new matter was added.

Reconsideration of the present application is respectfully requested.

Objection to Specification

The Examiner has objected to the specification because it refers to catechin as a flavanol while a Phytochemical Dictionary from Harborne et. al., cited by the Examiner, defines catechin as a flavonoid. The Examiner believes that it is therefore unclear what class of compounds Applicants are referring to.

Applicants respectfully submit that flavonoids are simply a larger class of compounds that encompass flavanols (*see* Attachment). Referring to page 1 of the Attachment, the foundational structure of flavonoids is a flavan nucleus, upon which variations such as the number and position of OH and/or oxygen groups and/or double bonds occur to define the various subclasses of flavonoids. For example, the subclass, flavanol contains an OH group attached to the 3 position of the flavan nucleus, and it has no double bond in the C ring (*see* Attachment, pp. 3-4). Thus, flavanols are a subclass of flavonoids. Applicants have shown by the use of a structural formula what is understood as "flavanol" (*see* specification, page 5, lines 17-21).

Therefore, withdrawal of the objection is respectfully requested.

Rejection under 35 U.S.C. §112, 2nd Paragraph

Claims 1-18 and 29-32 are rejected as indefinite under 35 USC § 112, second paragraph on the same ground as the Examiner's objection to the specification. Based on Applicants' above remarks, withdrawal of the rejection is believed to be in order. Such action is respectfully requested.

Rejections under 35 U.S.C. § 112, 1st Paragraph

The Examiner rejected claims 1-18 and 29-32 for lack of written description under 35 USC § 112, first paragraph on the ground that the specification fails to teach how to assess individual baseline cytokine levels in order to diagnose or treat an individual. Applicants respectfully traverse the rejection.

The essence of Applicants' invention is the discovery of a diagnostic tool that allows for individualized (personalized) diagnosis. Series of flavanols and procyanidins is used to diagnose TGF-β responsiveness of a subject. Depending on the starting body levels of TGF-β, the subject will respond differently to various flavanols and procyanidins as detailed below. It is that differential response that is used for diagnosis.

Thus, the specification defines "baseline cytokine level" (page 12, lines 13-16) and teaches that a subject's "baseline cytokine level" can be obtained by extracting a body sample, such as blood, and measuring the level of cytokines in the sample as described, for example in Example 2, pages 26-30. To determine "cytokine responsiveness," aliquots of the sample are incubated with a series of flavanols and procyanidins ranging from monomers *e.g.*, epicatechin to procyanidin decamers, or mixtures thereof, and the resulting TGF- β levels are measured following incubation (*see e.g.*, page 12, line 17-page 13, line-2 of the specification).

By comparing TGF-β levels after incubation of each diagnostic sample with the baseline TGF-β level (*i.e.*, prior to incubation), and determining whether those values have increased or decreased, a subject can be diagnosed as a low or a high baseline TGF-β producer (page 13, line 3 to page 14, line 2). For example, if a subject shows an

increase of TGF-β in an epicatechin and lower oligomer sample in comparison with the baseline levels, this subject is diagnosed as a low baseline producer. On the other hand, if a subject shows a decrease in TGF-β in higher oligomer procyanidin samples in comparison with the baseline levels, this subject is diagnosed as a high baseline producer (page 13, lines 6-18 and Example 2, *see also* Figures 2 and 3).

Once a subject is diagnosed as a low or high TGF-β producer, a person of skill in the art would know to identify which condition(s) the subject suffers from, or more importantly is at risk of, given known correlations between TGF-β and certain medical conditions such as atherosclerosis, cardiac fibrosis, coronary heart disease and renal disease or failure (*see*, specification page 14, line 19 to page 15, line 18). Once diagnosed, dietary and pharmaceutical regimens can be designed, and a subject may be treated, as described in the specification (e.g. using flavanol/procyanidin compounds) or any known method to increase or decrease TGF-β (*see* page 16, line 4-page 23, line 6).

Thus, a person of skill in the art would know how to diagnose a subject as a low or high baseline producer based on the above summarized guidance in the specification, and would then know how to treat the subject based on the guidance in the specification and knowledge in the art.

In summary, in view of the guidance in the specification and the general knowledge in the art, a person of skill in the art would have recognized that Applicants were in possession of the invention as of the filing date of the application. Withdrawal of the rejection is respectfully requested.

* * *

The Examiner sites several grounds for rejection of claims 1-19 and 29-32 for lack of enablement under 35 USC § 112, first paragraph. Applicants will address them in turn.

(i) The Examiner states that only claims directed to TGF-β are enabled. The claims have been amended to recite TGF-β. Therefore, the rejection is now moot.

(ii) The Examiner states that only the effect of TGF-β on PBMC is enabled. Applicants respectfully traverse the rejection. Applicants need not enable *all* cells in the body of a human or veterinary animal because a person of skill in the art would know

which cells express TGF-β and thus would know how to select a suitable body sample to use in the methods of claims 1-19 and 29-32. Withdrawal of the rejection is respectfully requested.

(iii) The Examiner also asserts that the specification does not provide enablement for derivatives of compounds recited in the rejected claims. Claims 7, 13 and 15 were amended to delete reference to derivatives. Therefore, the rejection is now moot.

(iv) Further, the Examiner states that the claims lack enablement because they recite "homeostatic cytokine levels" while the specification does not indicate what constitutes a homeostatic cytokine level. While Applicants believe that homeostasis is a recognized term in the medical art, and is defined in the specification at page 13, lines 25-27, to expedite the prosecution of the application, the term has been rephrased. Withdrawal of the rejection is respectfully requested.

(v) Lastly, the Examiner rejected claims directed to treatment of diseases as lacking enablement because the specification contains no examples of treating diseases. As was disclosed at pages 14-15 in the specification, specific diseases related to varying levels of TGF-β as well as other cytokines were well known in the art as of the effective filing date of the application. For example, decreased levels of TGF-β1 had been detected in subjects with advanced atherosclerosis (*see* Baxter et al., J Cardiovasc Pharm 2001;38:930-939), excess TGF-β1 has been shown to lead to cardiac fibrosis (*see* Pearson et al., Methods Enzymol 2001;335:350-360), renal disease or failure, coronary heart disease (*see* Grainger et al., Hu Mol Gen 1999;8:93-97) and coronary vasculopathy following cardiac transplantation (*see* Wang et al., Cardiovasc Res 1997;34:404-410). Based on the guidance in the specification and the general knowledge in the art, a person of skill in the art would have been able to identify conditions that can benefit from reducing or increasing TGF-β levels. Withdrawal of the rejection is respectfully requested.

Conclusion

In view of the above amendment and remarks, Applicants believe that the application is in condition for allowance. Such action is respectfully requested.

Respectfully submitted,

Date: May 25, 2006

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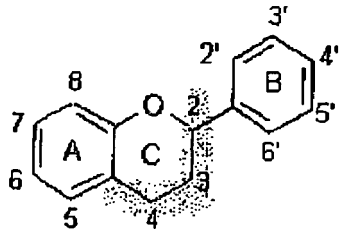
The Phytochemistry of Herbs

Flavonoid Antioxidants

For further information, see Phenolics Advanced page

Flavonoids, the most abundant polyphenols in the diet, can be classified into ten groups based on differences in their chemical structures. The intermediate page will use chemical structures this month, because it's hard to understand the differences between flavonoids without seeing a picture. I've tried to explain the "hieroglyphs" in the text accompanying them for herbalists who haven't studied organic chemistry. See the glossary for definitions as needed.

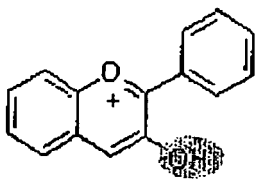
On the left below is the basic "flavan nucleus," the foundation structure upon which flavonoids are constructed. If you look at all the flavonoid structures, you will see that they have this pattern, or a variation of it, in common.



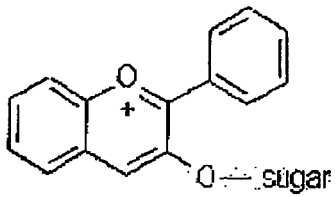
In this map of a chemical structure, the point of each angle represents a carbon atom. The lines between the points show chemical bonds between adjacent atoms. The 'A' ring and the 'B' ring are made of six carbon atoms each which are bonded together to form a special structure known as an aromatic ring. The numbers next to each point are called "positions" on this structure. At each position is a carbon atom where specific small groups of atoms called functional groups may attach. The A ring and the B ring are attached to each other by a "three-carbon bridge" (shaded area). This bent bridge, along with an oxygen atom, makes up the 'C' ring.

The different classes of flavonoid structures are distinguished by fairly minor variations on this pattern. Below are the basic structures of eight of the different classes. Within each of these classes, there are many further variations on the theme. Some examples are given after the basic skeletons of each class.

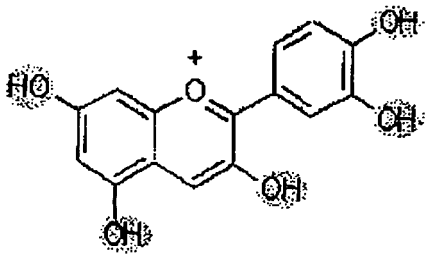
Anthocyanidins and Anthocyanins: These molecules are very similar to the flavan nucleus above. One difference is that the oxygen atom has a positive charge on it; there are also two double bonds in the C ring. There are many different kinds of anthocyanidins and anthocyanins, varying in the number and position of -OH groups, sugar groups, and other functional groups attached. Some are quite complicated, with parts of other flavonoid molecules attached to them. This class of flavonoids contains the pigments that give certain fruits, vegetables and herbs their dark red, blue, and purple colors. Many of them are antioxidants.



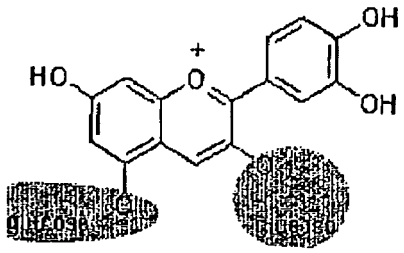
Anthocyanidin (aglycone). This kind of molecule lacks any attached sugars. Notice the -OH group at position 3. Various anthocyanidins have -OH groups at other positions on both the 'A' and 'B' rings.



Anthocyanin (glycoside). In this skeleton, the -OH group at position 3 has been replaced by one (or more) sugar molecules. In some anthocyanins, there are additional sugar groups attached at other positions as well.



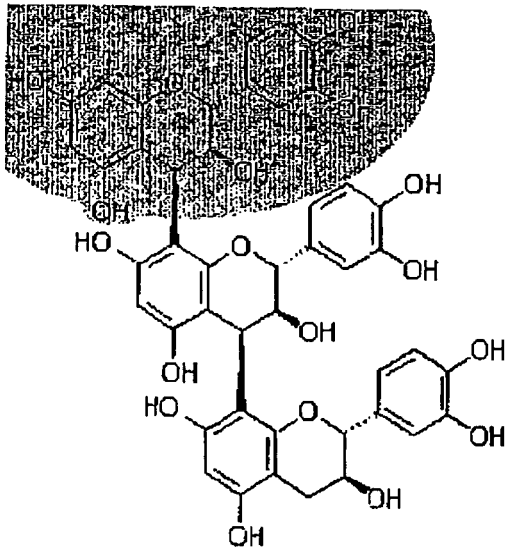
This is a particular type of anthocyanidin aglycone known as *Cyanidin*. It's composed of the basic anthocyanidin skeleton with four more -OH groups attached at positions 5, 7, 4' and 5'. *Cyanidin* is found in Grapes, Bilberry, Blueberries, Black Cherry, Cocoa powder, and many other medicinal herbs and foods.



This is the glycoside of *cyanidin*, known as *Cyanin*. It differs from *Cyanidin* by having glucose molecules replace the -OH groups at positions 3 and 5. It is found in Elderberry and many of the same plants that contain cyanidin.

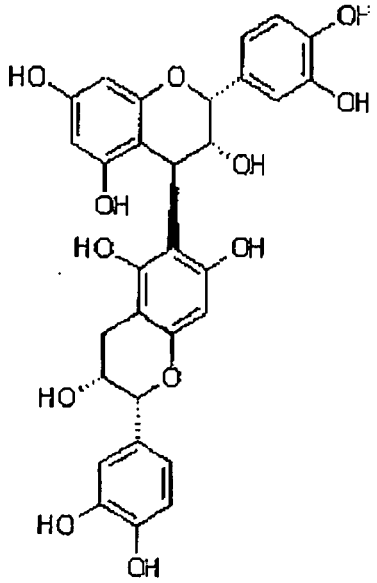
Proanthocyanidins: This group of important antioxidants contains polymers made from multiple anthocyanidin-like molecules, known as flavanols (see below). They are called proanthocyanidins because, if broken apart with acid treatment,

proanthocyanidins yield anthocyanidins such as *Cyanidin*. Proanthocyanidin polymers consisting of two to ten or more subunits have been identified. Oligomeric proanthocyanidins (OPCs) are the water-soluble, short-chain polymers. Proanthocyanidins are sometimes referred to as "condensed tannins" and are responsible for astringency in many foods and medicinal herbs. Red wine contains many complex proanthocyanidins (extracted from grape skins and seeds); so do blueberries, blackberries, strawberries, elderberries, and other red/blue/purple colored plant parts.



This is a proanthocyanidin composed of three linked subunits. The shaded area represents one subunit, which is a flavanol known as *Catechin* (see below). This proanthocyanidin is called *Procyanidin C2*.

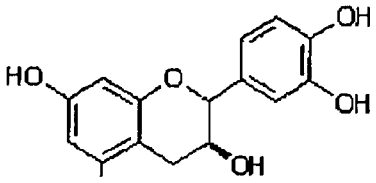
(Why are some bonds represented as Wedges?).



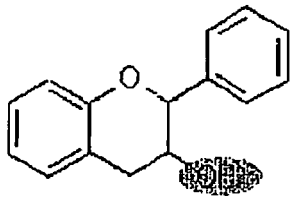
Here is *Procyanidin B5*, consisting of two subunits linked 'sideways' compared to the architecture of *Procyanidin C2*.

Some of the more complex proanthocyanidins contain subunits linked in both ways. Some phytochemists believe that the larger, yet-to-be-identified molecules in complex substances such as aged red wine could have fifty or more of these linked subunits.

Flavanols: Let's look at a particular type of flavanol known as a **flavan-3-ol**, which has an -OH group attached to the 3 position of the basic flavan skeleton. The "-ol" ending comes from the word "alcohol" which generally means "an organic molecule with an -OH group attached (i.e., ethanol)." Flavan-3-ols are the subunits of proanthocyanidins. Their structures are very similar to those of anthocyanidins, except that there is no positive charge on the oxygen atom and no double bonds in the C ring.



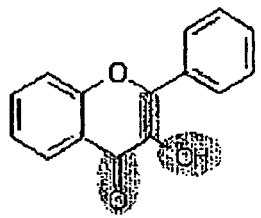
This is *Catechin*, a common flavan-3-ol that occurs in many plants. It's found in Green tea, Cocoa powder,



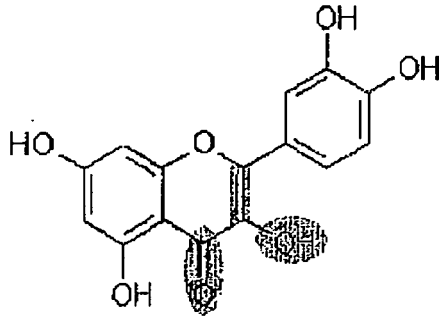
Here is the basic flavan-3-ol skeleton. It's just a flavan nucleus with an -OH group attached to position 3 of the C ring.

Red wine, Hawthorn, Bilberry, Motherwort, and other herbs. It is also a common subunit of proanthocyanidin polymers such as *Procyanidin C2* above. *Epicatechin* is another common example; it differs from *Catechin* only in the spatial orientation of its -OH group.

Flavonols: Notice this word is spelled with an "o" instead of with an "a" as in "flavanols". This means that the molecule has a double-bonded oxygen atom attached to position 4. They're still "-ols" because they retain the -OH group at position 3 like the flavanols; but they also have a double-bonded oxygen atom, which makes them like another class of flavonoids known as "flavones" (see below).

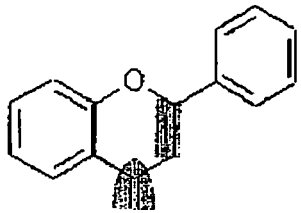


This is the basic flavonol skeleton, with the -OH at position 3 and the =O at position 4. It also differs from flavanols by having a double bond between carbons 2 and 3 on the C ring.

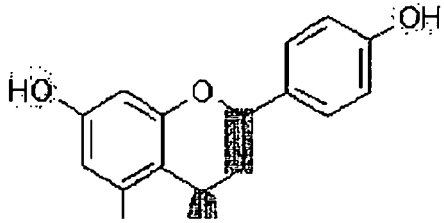


Here's the common flavonol, *Quercetin*. It's the most abundant flavonol in the diet and is found in hundreds of herbs and foods. Onions are especially rich in *Quercetin*. It has proven antioxidant effects.

Flavones: Flavones are like flavonols, without the "-ol." In other words, there is no longer an -OH group at position 3 on the central ring.



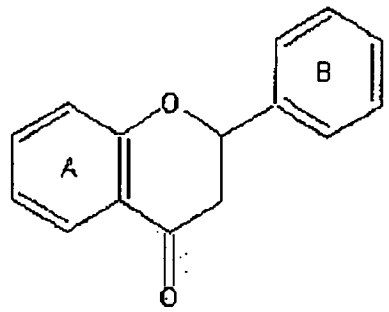
Here's the basic flavone skeleton, with the =O at position 4 and the double bond between carbons 2 and 3.



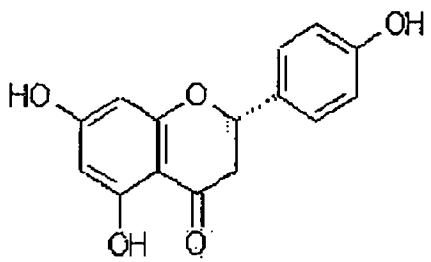
This is *Apigenin*, a flavone with -OH groups added to positions 5, 7, and 4'. It's another very

common flavonoid, appearing in many medicinal plants and foods such as celery. Another flavone is luteolin, found in sweet red peppers.

Flavanone: Take away the double bond between carbons 2 and 3 of the flavone structure, and you have a flavanone. Notice the "o" has changed back to an "a," which indicates that the flavanones have a single bond between carbons 2 and 3, like the basic flavan nucleus at the top of this page.

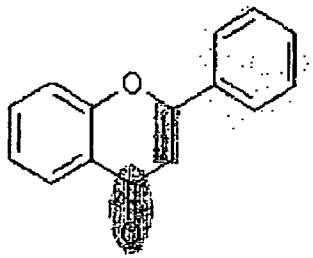


The basic flavanone skeleton retains the =O, which makes it an "-one." Many flavanones occur as glycosides; for example, hesperitin (aglycone) and hesperidin (glycoside) occur in citrus along with naringenin (--->)

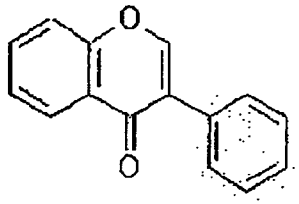


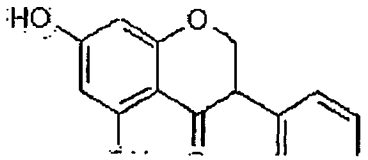
Naringenin, an antioxidant flavanone from citrus species, has -OH groups attached at positions 5, 7, and 4'. Studies have indicated that it has anti-inflammatory, anti-cancer, and liver protective effects.

Isoflavones: Isoflavones (also known as isoflavonoids) are very similar to flavones, except the B ring is attached to position 3 of the C ring, rather than to position 2 as in the flavones:

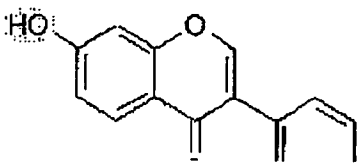


To the left is the basic flavone skeleton, with the B ring attached to position 2 of the central ring. On the right is the isoflavone skeleton, which is exactly the same as the flavone skeleton but with the B ring attached to position 3. "Iso" is short for "isomer."





Here is the isoflavone *Genistein*, found in Red clover, Alfalfa, Peas, Soy



This isoflavone, *Daidzein*, is very similar to *Genistein*, only

& other legumes. It consists of the basic isoflavone skeleton with -OH groups attached at positions 5, 7, and 4'. *Genistein* is protective against breast, prostate, and colon cancers and can help with hot flashes and osteoporosis prevention.	lacking the -OH group at position 5. It's found in the same kinds of plants as *Genistein* and acts in much the same way. Both of these isoflavones are anti-inflammatory and show cardioprotective and mild antioxidant activities.
For in-depth information on flavonoids in berries, see this interesting doctoral dissertation from Finland.	

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